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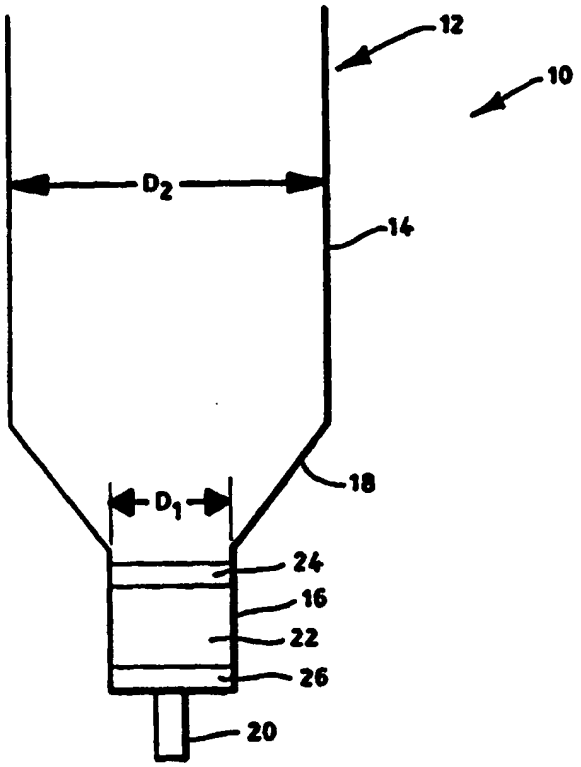
(54) Title: DUAL STAGE INTERNAL DIAMETER IN SOLID PHASE EXTRACTION WELL

(57) Abstract

An assay well (10) provides upstanding wells (12) with at least two cylindrical segments (14, 16) of differing diameter (D1, D2) connected by a transition zone (18) of tapering cross section. The wells (10) permit a wide range of selectable configurations by placing frits (24, 26) which sandwich medium (22) therebetween at different elevations within the well (10) depending on the intended use of the configuration. Depending on the elevation of the frits (24, 24', 24'', 26, 26', 26'') the volume of medium (22, 22', 22'') sandwiched therebetween may be selectably varied.



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DUAL STAGE INTERNAL DIAMETER IN SOLID PHASE EXTRACTION WELL

Field of Invention

The present invention relates generally to solid phase extraction (SPE) techniques, and more specifically to an assay well for chemical, biological, or biochemical substance purification or enrichment for analysis.

Background of Invention

Substance analysis may be carried out in an assay tray having a plurality of wells for batch processing or analysis, each of the wells receiving a sample of solution or analyte. Presently, a 96-well assay tray where the wells are arranged in an 8 x 12 matrix has found increasing acceptance in chemical and pharmaceutical research. Assay trays for analysis typically comprise a well for receiving a sample of solution through an open top end. The solution is then allowed to percolate by the force of gravity, or by negative or positive pressure, downward to a delivery opening or spout. Intermediate to the delivery opening and the open top is a separation medium, or sorbent, which promotes the separation of certain analytes or solids from the solution. Based on the experimental parameters of interest and the exact solution character involved, the separation medium varies in composition, character, and amount.

Solid phase extraction (SPE) is a process by which liquid samples may be purified or enriched for analysis and is useful for a broad range of applications. As mentioned hereinabove, different applications may require different SPE configurations. For example, in the analysis of drugs from biological fluids, 5-100 mg of SPE sorbent may be typically required to extract analyte from 1 ml of plasma. Environmental applications, such as the analysis of pesticides from groundwater, may require several grams of sorbent to process liters of samples. Because there are such diverse applications, no single SPE configuration may be expected to be ideal for all possible uses. Typically, commercial vendors of SPE apparatus make a number of configurations and formats available. For example, syringe barrel cartridges are typically manufactured in several different barrel sizes, ranging from 1cc to 35cc, with sorbent masses ranging from 30 mg to 10 g. The obvious disadvantage to manufacturing such a wide variety of sizes is that different molds must be produced for each of the configurations sold, leading to increased expenses associated therewith.

In the commercial and research spheres of bioanalysis, the 96-well SPE assay tray, or plate, is being increasingly accepted. Some manufacturers sell a basic configuration 96-well plate containing approximately 30-100 mg of sorbent in each well. For many of the applications for which this plate is used, 30 mg is significantly more than required to adsorb the analytes of interest. The excess sorbent is justified by the costs of manufacturing saved by providing a plate which will accommodate many uses, but is basically an excess necessary to provide reproducible analytical results. A disadvantage of this current approach is that the end-user when employing the standard-configuration plate is compelled to process with more elution solvent than is necessary for some small-volume analytical procedures because of the dimensions of the wells of the plate.

An assay tray assembly manufactured by Porvair Corporation has a plurality of right cylindrical spigots accommodating sorbent between two frits. The diameter of each well is approximately 7mm. A 30 mg sorbent bed has a height of approximately 3mm in the spigot. While it is possible to reduce the sorbent mass in the Porvair plate to 15 mg and still achieve acceptable performance, it is not possible to decrease the sorbent mass much below 15 mg in the current well ID configuration, as it will be difficult to consistently manufacture a uniformly packed bed. It is well known that variations in bed uniformity can lead to decreased performance resulting in greater error.

Others have avoided the problem of consistent manufacture of a packed bed of sorbent by providing a substantially tapered bore inside the well, instead of the right cylindrical spigot of the Bojanic patent. U.S. Patent No. 5,035,866 to Wannlund provides a tapered well which may hold a solid reactant, such as a luminescent enzyme. The disadvantage of such a tapered shape is that the reactant, or analogously the sorbent, must be formed in situ. While this works well for the molded reagent of Wannlund, it is problematic in the case of pulverulent, slurry, colloidal, or other non-solid forms of sorbent, which requires the fitting of frits against the tapered wall. The result is that the frits might not securely retain the separation medium. The frictional fit of the upper frit is compromised by the sloping internal diameter of the well wall. Deformation of the frit is also possible as it is pressed into the tapered well.

Currently, prior art SPE plates are not capable of effectively accommodating a broad range of sorbent medium masses within the well. As set forth hereinabove, too wide a diameter results in poor reproducibility during

manufacturing, while too small a diameter can result in excessively high back pressure or low flow rates at a constant pressure during use. One way to increase the range of sorbent masses would be to create several molds, each containing a different ID in each well. However, it is quite expensive to manufacture a mold for a 96-well plate, that can accommodate a broad range of sorbent masses

Summary of Invention

The present invention provides a flexibly configurable well plate that selectably provides a broad range of medium or sorbent masses with a high degree of bed uniformity.

According to the invention, an assay plate has upstanding, preferably tubular wells with at least two slightly tapered cylindrical segments of differing diameter connected by a transition zone having a more substantially tapered cross section. The wells permit several configurations using frits disposed in the cylindrical segment(s) or transition zone which sandwich sorbent or separation medium therebetween at different elevations within the well. Depending on the elevation of the frits, the volume sandwiched therebetween may be selectably varied.

The present invention advantageously provides for a low sorbent mass configuration, an intermediate sorbent mass configuration and a high sorbent mass configuration. In the low sorbent mass configuration a bottom portion of the well below a transition zone, which is described by a first smaller ID, houses sorbent. In the intermediate sorbent mass configuration, a top portion of the well above a transition zone, which is described by a second larger ID, houses the sorbent. In the large sorbent mass configuration, the top and bottom portions of the well including the transition zone house the sorbent.

Features of the invention include a large number of possible configurations as a function of the elevation(s) of frits and the volume(s) circumscribed thereby. Further, the present invention provides an assay test well which may be produced relatively inexpensively, for either one-time or repeated use. The well may advantageously be provided in a form which is adaptable to mechanical or automated handling, pipetting of analyte, and removal of percolate or eluate for further analysis or processing.

Brief Description of Drawings

These and other features and advantages of the present invention will become more apparent in view of the following detailed description in conjunction with the accompanying drawing, of which:

FIG. 1 is a cross sectional view of a low sorbent or medium mass configuration according to one aspect of the instant invention;

FIG. 2 is a cross sectional view of an intermediate sorbent or medium mass configuration according to another aspect of the instant invention;

FIG. 3 is a cross sectional view of a high sorbent or medium mass configuration according to still another aspect of the instant invention.

Detail Description Of An Illustrative Embodiment

Referring to the Figures, a cross sectional view of an illustrative SPE analytical testing assembly 10 is shown. The testing assembly 10 comprises at least one well 12, and may comprise any convenient number and in any desired configuration such as desired to accommodate various testing procedures. For example, a single tube for handling in a carousel is envisioned, as well as a 96-well array as is currently gaining acceptance in the art. The wells 12 in this illustrative embodiment are comprised of at least three chambers which form a composite chamber, including an upper chamber 14, a lower chamber 16, and a transition zone 18. The upper and lower chambers are preferably configured with only a slight taper, on the order of ½ degree, in order to facilitate easier mold release during manufacture. Typically, the upper end of the upper chamber is open to permit the introduction of analyte and provides a reservoir for holding a quantity of analyte, and the lower end of the lower chamber is provided with a spout 20. Lower chamber 16 has a major diameter D1, and upper chamber 14 has a major diameter D2, where D2 is greater than D1.

In the cross sectional view of Fig. 1, an illustrative SPE analytical testing assembly 10 is configured for low sorbent mass applications. Sorbent 22 is accommodated in the lower chamber 16, and is sandwiched by two frits including an upper frit 24 and a lower frit 26 corresponding each to diameter D1. The frits may be made of any suitable material which retains the sorbent 22 and is permeable by an analyte to the extent desirable for a given use. One material frequently employed is sintered polyethylene. The sorbent, as known in the art, will be selected according to the assay being performed. It is envisioned

that the elevation of at least the upper frit 24 may be varied to define an adjustable volume of sorbent 22 between the frits 24, 26.

FIG. 2 illustrates a cross sectional view of an illustrative SPE analytical testing assembly 10 configured for intermediate sorbent mass applications. The sorbent 22 is accommodated in the upper chamber 14, and is sandwiched by two larger frits 24', 26' corresponding each to diameter D2. In one embodiment, the lower frit 26' is supported by a plurality of supports 28 which provide stability but are not so large as to obstruct the passage of analyte exiting the sorbent 22. Again, in this embodiment it is envisioned that the elevation of at least the upper frit 24' may be varied to define an adjustable volume of sorbent 22 between the frits 24', 26'.

Referring now to FIG. 3, a cross sectional view of an illustrative SPE analytical testing assembly 10 is configured for high sorbent mass applications. In this configuration the sorbent 22 is accommodated in the lower chamber 16, the transition zone 18, and the upper chamber 14 and is sandwiched by two frits comprised of a larger upper frit 24" and a smaller lower frit 26". As seen in the figure, the upper frit 24" is of a diameter that corresponds to diameter D2, and the lower frit 26" to D1. Again, it is envisioned that the elevation of at least the upper frit 24" may be varied to define an adjustable volume of sorbent 22 between the frits 24", 26". The upper frit 24" may also rest on a boundary surface between the transition zone 18 and the upper chamber 14.

Although not illustrated, either of the frits can be placed in the transition zone 18, with the remaining frit in either the upper chamber 14 or the lower chamber 16.

According to an illustrative use of the disclosed embodiments, an analyte is introduced into the well 12 through the upper open end thereof and allowed to elute or percolate through the sorbent 22 and the frits. The analyte exiting the sorbent 22 passes eventually, by force of gravity or differential pressure, through the spout 12 and thereafter is captured for further processing. The well may be connected via the spout 20 to a vacuum chamber (not shown) or other apparatus for receiving the analyte through the sorbent.

As mentioned hereinabove, the assay well of the instant invention may be provided with a selected medium or sorbent, selected frits arranged so as to sandwich a selected amount of medium as discussed herein so that the wells

may be delivered rapidly and specifically for an intended assay. The present invention allows for a single plate to be manufactured without the need for separate molds, separate production runs of the molds, or separate inventorying of separate plates.

It should be appreciated that although the terms "sorbent" and "separation medium" are used in the present description, those terms are used substantially interchangeably and can be construed to include a chromatographic sorbent, or other medium such as solid-phase support for synthesis, or the like, as used in combinatorial chemistry, peptide synthesis, etc.

Although the frit disposed in the transition zone is in abutment with a plurality of tabs as described herein, it will be appreciated that other means of securing the frits are envisioned such as by friction fitting, any of various abutment surfaces, or the like.

Although the well described herein is comprised of an upper zone, a transition zone and a lower zone it will be appreciated that a plurality of upper zones and lower zones with intermediate transition zones can be configured according to the invention.

While the wells described in the embodiment herein include upper and lower chambers preferably configured with a slight taper on the order of ½ degree, in order to facilitate easier mold release during manufacture, it will be appreciated that a right cylindrical configuration or other geometrical configuration is possible.

Although the invention has been shown and described with respect to exemplary embodiments thereof, various other changes, additions and omissions in the form and detail thereof may be made therein without departing from the spirit and scope of the invention.

What is claimed is:

1. An assay well comprising:

a first chamber having a first open end and a second end and having a first inside diameter;

a second chamber having a first end and a second end in communication with a spout and having a second inside diameter;

a transition zone connecting said second end of said first chamber and said first end of said second chamber and having an inside diameter varying from said first inside diameter of said first chamber to said second inside diameter of said second chamber;

said first chamber, second chamber, and transition zone forming a composite chamber having an internal longitudinal dimension defining an elevation;

a first frit and a second frit; said frits being selectably placed at a first and a second elevation within said composite chamber and defining a volume therebetween;

a medium maintained in said volume defined by said first and second frits.

2. The assay well of claim 1, wherein said transition zone has a uniformly varying inside diameter.

3. The assay well of claim 1, wherein said first frit is proximate said spout in said second chamber, and disposed said second frit is also in said second chamber.

4. The assay well of claim 1, wherein said first frit is proximate said spout in said second chamber, and said second frit is disposed in said first chamber.

5. The assay well of claim 1, wherein said first frit is proximate said spout in said second chamber, and said second frit is disposed in said transition zone.

6. The assay well of claim 1, wherein said first frit is disposed in said transition zone, and said second frit is disposed in said first chamber.

7. The assay well of claim 1, wherein said first frit is disposed in said first chamber, and said second frit is disposed in said first chamber.

8. The assay well of claim 7, wherein said transition zone further comprises frit supports for supporting said first frit, said first frit being at an elevation closer to said spout than said second frit.

9. The assay well of claim 1, wherein said first inside diameter of said first chamber is greater than said second inside diameter of said second chamber.

10. The assay well of claim 9, wherein said transition zone has a uniformly varying inside diameter.

11. A method for making a testing assembly having at least one test well comprising the steps of:

configuring a first chamber having a first open end and a second end and having a first inside diameter;

configuring a second chamber having a first end and a second end and having a second inside diameter;

providing a transition zone connecting said second end of said first chamber and said first end of said second chamber and having a varying inside diameter ranging from said first inside diameter of said first chamber to said second inside diameter of said second chamber, said first chamber, second chamber, and transition zone forming a composite chamber having an internal longitudinal dimension defining an elevation;

selectively placing a first frit and a second frit at a first and a second elevation within said composite chamber and defining a volume therebetween;

placing a medium in said volume defined by said first and second frits.

12. The method of claim 11, wherein said step of selectively placing said frits further comprises placing said first frit proximate to a spout at said second end in said second chamber, and placing the second frit in the second chamber.

13. The method of claim 11, wherein said step of selectively placing the frits further comprises placing said first frit proximate the spout in said second chamber, and placing said second frit in said first chamber.

14. The method of claim 11, wherein said step of selectively placing said frits further comprises placing said first frit is proximate a spout at said second end in said second chamber, and placing said second frit in said transition zone.

15. The method of claim 11, wherein said step of selectively placing said frits further comprises placing said first frit in said transition zone, and placing said second frit in said first chamber.

16. The method of claim 11, wherein said step of selectively placing said frits further comprises placing said first frit is in said first chamber, and placing said second frit in said first chamber.

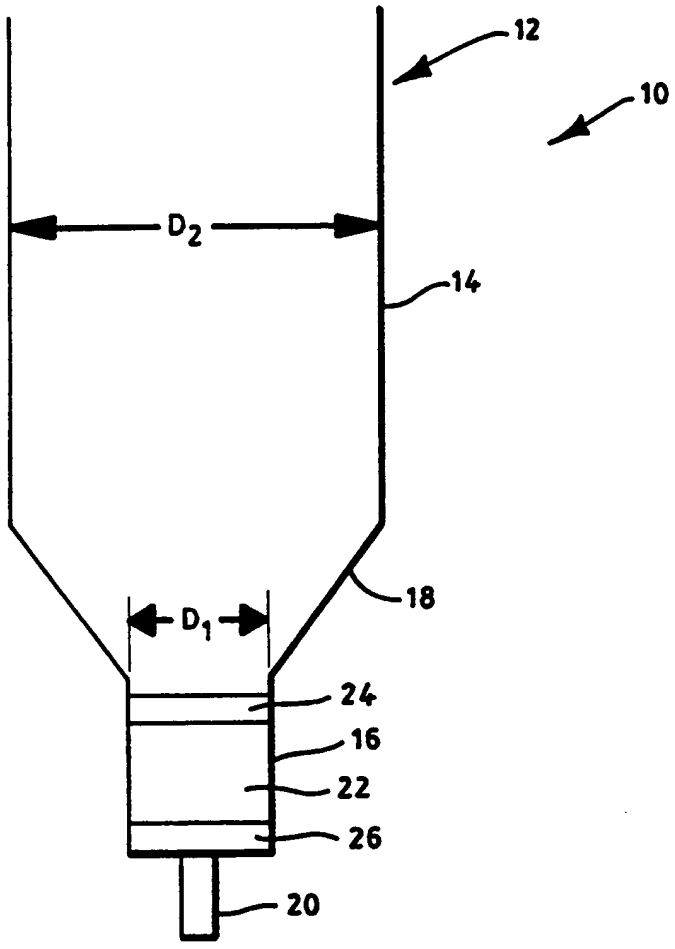

FIG. 1

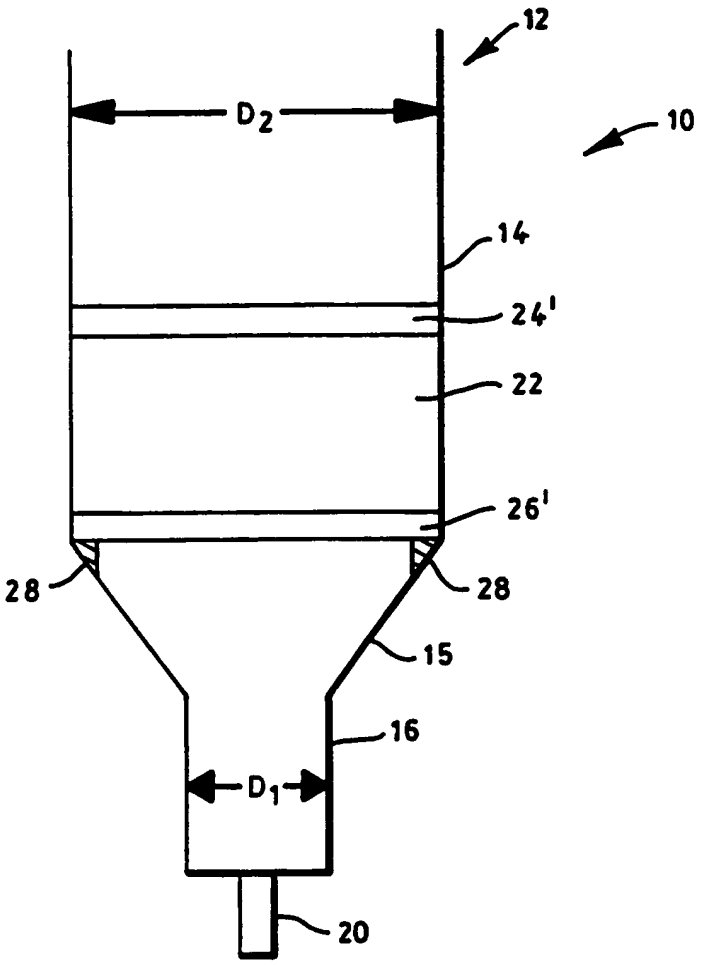

FIG. 2

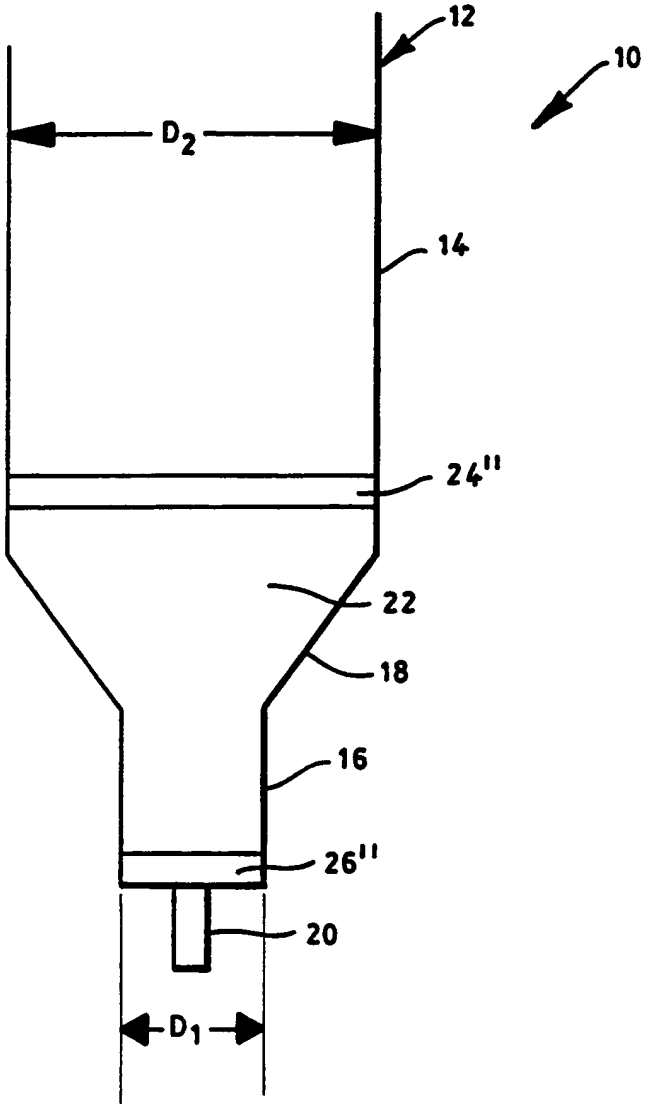

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 30/60; B01D 15/08
US CL :210/198.2, 282; 422/59, 60, 69, 70, 99, 101

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 210/198.2, 282; 422/59, 60, 69, 70, 99, 101

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 4,787,971 A (DONALD) 29 November 1988, see entire document.	1-5,9-14 --- 6-8,15-16
X	US 4,775,629 A (KUHL et al) 04 October 1988, see entire document.	1-2,6-7,9-1 1,15-16
X --- Y	US 5,037,544 A (SNYDER) 06 August 1991, see entire document.	1-3,5,9-12,14 --- 8
X	US 4,151,254 A (GIMOVSKY) 24 April 1979, see entire document.	1-3,9,11-12
X	US 4,254,082 A (SCHICK et al) 03 March 1981, see entire document.	1-3,9-12

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01132

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIO-RAD Product Catalog, March 1988, page 86, see the Econo-Pac 10 Disposable Columns section.	1-3,9-12
Y	FISHER Product Catalog, 1988, pages 258-259, see entire document.	1-16
Y	US 4,341,635 A (GOLIAS) 27 July 1982, see entire document.	1-16
Y	US 5,585,068 A (PANETZ et al) 17 December 1996, see entire document.	1-16
Y	SUPELCO Product Catalog, 1996, pages 359-365, see entire document.	1-16
A	US 3,567,029 A (QUAME) 02 March 1971.	1-16